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DATE: Wednesday, April 20, 2005

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☐	L2	L1 same (combinatorial or (peptide near2 library))	179
☐	L1	(culture or growth) adj2 (media or medium)	120989

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                   data from INPADOC
 NEWS  5  FEB 28   BABS - Current-awareness alerts (SDIs) available
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 NEWS  7  MAR 02   GBFULL: New full-text patent database on STN
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=> file .pub
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FULL ESTIMATED COST                                     0.21       0.21
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FILE 'BIOSIS' ENTERED AT 14:11:12 ON 20 APR 2005
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=> s (culture or growth)(2w) (media or medium)
L1      192006 (CULTURE OR GROWTH)(2W) (MEDIA OR MEDIUM)

=> l1 same (combinatorial or library)
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=> s l1 same (combinatorial or library)
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nested terms that are not separated by a logical operator.

=> s l1 and (combinatorial or library)
L2        1110 L1 AND (COMBINATORIAL OR LIBRARY)

=> s l2 and peptide
L3         205 L2 AND PEPTIDE

=> duplicate remove l3
DUPLICATE PREFERENCE IS 'MEDLINE, BIOSIS'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L3
L4          148 DUPLICATE REMOVE L3 (57 DUPLICATES REMOVED)

=> d 1-5 bib ab

L4   ANSWER 1 OF 148     MEDLINE on STN
AN   2005083964     MEDLINE
DN   PubMed ID: 15591139
TI   Gene structure of the goldfish agouti-signaling protein: a putative role
     in the dorsal-ventral pigment pattern of fish.
AU   Cerda-Reverter Jose Miguel; Haitina Tatjana; Schioth Helgi Birgir; Peter
     Richard Ector
CS   Department of Fish Reproductive Physiology, Instituto de Acuicultura de
     Torre de la Sal, Consejo Superior de Investigaciones Cientificas, 12595
     Torre de la Sal, Ribera de Cabanes, Castellon, Spain..
     cerdarev@iats.csic.es
SO   Endocrinology, (2005 Mar) 146 (3) 1597-610.  Electronic Publication:
     2004-12-09.
     Journal code: 0375040. ISSN: 0013-7227.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Abridged Index Medicus Journals; Priority Journals
EM   200504
ED   Entered STN: 20050217
     Last Updated on STN: 20050405
     Entered Medline: 20050404
AB   One of the most successful chromatic adaptations in vertebrates is the
     dorsal-ventral pigment pattern in which the dorsal skin is darkly colored,
     whereas the ventrum is light.  In fish, the latter pattern is achieved
     because a melanization inhibition factor inhibits melanoblast
     differentiation and supports iridophore proliferation in the ventrum.  In
     rodents, the patterned pigmentation results from regional production of
     the agouti-signaling protein (ASP).  This peptide controls the
     switch between production of eumelanin and pheomelanin by antagonizing
     alphaMSH effects on melanocortin receptor (MCR) 1 in the melanocytes.  In
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addition, ASP inhibits the differentiation and proliferation of melanoblast. Thus, the mammalian ASP may be homologous to the poikilotherm melanization inhibition factor. By screening of a genomic **library**, we deduced the amino acid sequence of goldfish ASP. The ASP gene is a four-exon gene spanning 3097 bp that encodes a 125-amino acid precursor. Northern blot analysis identified two different ASP mRNAs in ventral skin of red- and black-pigmented and albino fish, but no expression levels were observed in the dorsal skin of the same fish. The dorsal-ventral expression polarity was also detected in both black dorsally pigmented fish and albino fish. Pharmacological studies demonstrate that goldfish ASP acts as a melanocortin antagonist at Fugu MC1R and goldfish MC4R. In addition, goldfish ASP inhibited Nle4, D-Phe7-MSH-stimulated pigment dispersion in medaka melanophores. Our studies support agouti signaling protein as the melanization inhibition factor, a key factor in the development of the dorsal-ventral pigment pattern in fish.

L4 ANSWER 2 OF 148 MEDLINE on STN
AN 2005172193 IN-PROCESS
DN PubMed ID: 15806288
TI Identification of **Peptides** Inhibiting Adhesion of Monocytes to the Injured Vascular Endothelial Cells through Phage-displaying Screening.
AU Guo Yu; Zhang Jia; Wang Ji-Cheng; Yan Feng-Xiang; Zhu Bing-Yang; Huang Hong-Lin; Liao Duan-Fang
CS Institute of Pharmacy and Pharmacology, Nanhua University, Hengyang 421001, China.. dfliao66@yahoo.com.cn
SO Acta Biochim Biophys Sin (Shanghai), (2005 Apr) 37 (4) 227-33. Journal code: 101206716. ISSN: 1672-9145.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20050405
Last Updated on STN: 20050405
AB Using oxidized low-density lipoprotein (LDL)-injured vascular endothelial cells (ECs) as target cells, **peptides** specifically binding to the injured ECs were screened from a phage-displaying **peptide library** by using the whole-cell screening technique after three cycles of the adsorption-elution-amplification procedure. Positive phage clones were identified by ELISA, and the inserted amino acid sequences in the displaying **peptides** were deduced from confirmation with DNA sequencing. The adhesion rate of ECs to monocytes was evaluated by cell counting. The activity of endothelial nitric oxide synthase (eNOS), and the expression levels of caveolin-1 and intercellular adhesion molecule-1 (ICAM-1) were determined by Western blotting. Six positive clones specifically binding to injured ECV304 endothelial cells were selected from fourteen clones. Interestingly, four phages had **peptides** with tandem leucine, and two of these even shared an identical sequence. Functional analysis demonstrated that the YCPRYVRRKLENELLVL **peptide** shared by two clones inhibited the expression of ICAM-1, increased nitric oxide concentration in the **culture media**, and upregulated the expression of caveolin-1 and eNOS. As a result, the adhesion rate of monocytes to ECV304 cells was significantly reduced by 12.1%. These data suggest that the anti-adhesion effect of these novel **peptides** is related to the regulation of the caveolin-1/nitric oxide signal transduction pathway, and could be of use in potential therapeutic agents against certain cardiovascular diseases initiated by vascular endothelial cell damage.

L4 ANSWER 3 OF 148 MEDLINE on STN
AN 2005084263 IN-PROCESS
DN PubMed ID: 15713888
TI Cleavage of apolipoprotein E by membrane-type matrix metalloproteinase-1 abrogates suppression of cell proliferation.

AU Aoki Takanori; Sato Daisuke; Li Yingyi; Takino Takahisa; Miyamori Hisashi; Sato Hiroshi
CS Department of Molecular Virology and Oncology, Cancer Research Institute, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan.
SO Journal of biochemistry, (2005 Jan) 137 (1) 95-9.
Journal code: 0376600. ISSN: 0021-924X.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20050217
Last Updated on STN: 20050304
AB Apolipoprotein E (apoE) in a human fetal brain cDNA **library** was identified, using the expression cloning method, as a gene product that formed a complex with latent matrix metalloproteinase (MMP)-2. Co-expression of membrane-type MMP-1 (MT1-MMP) with apoE in HEK293T cells reduced the amount of apoE secreted into the **culture medium**, whereas cell-associated apoE core protein was not affected. Incubation of native apoE protein with recombinant MT1-MMP resulted in the cleavage of apoE. Recombinant apoE protein fused to glutathione S-transferase (apoE-GST) was cleaved by MT1-MMP at the following **peptide** bonds; T(85)-M(86), K(93)-S(94), R(246)-L(247), A(255)-E(256) and G(296)-L(297). HT1080 cells transfected with the apoE gene, which express endogenous MT1-MMP, secreted a low level of apoE protein and its cleaved fragments, and treatment with MMP inhibitor BB94 induced accumulation of apoE and retardation of cell proliferation. Addition of apoE-GST protein to the culture of HEK293T cells suppressed cell proliferation, and stable transfection of the MT1-MMP gene partly abrogated the suppression. These results suggest that cleavage of apoE protein by MT1-MMP abrogates apoE-mediated suppression of cell proliferation.

L4 ANSWER 4 OF 148 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
AN 2004:323415 BIOSIS
DN PREV200400325214
TI **Peptides** for use in **culture media**.
AU Haaland, Perry D. [Inventor, Reprint Author]; Sherman, Douglas B. [Inventor]; Campbell, Robert L. [Inventor]; Stewart, Walter William [Inventor]; Lloyd, Sheila A. [Inventor]; Erickson, Bruce Wayne [Inventor]
CS Durham, NC, USA
ASSIGNEE: Becton, Dickinson and Company
PI US 6759510 July 06, 2004
SO Official Gazette of the United States Patent and Trademark Office Patents, (July 6 2004) Vol. 1284, No. 1. http://www.uspto.gov/web/menu/patdata.html . e-file.
ISSN: 0098-1133 (ISSN print).
DT Patent
LA English
ED Entered STN: 21 Jul 2004
Last Updated on STN: 21 Jul 2004
AB The present invention provides **peptides libraries** which are useful for rapid identification of biologically active compounds. The invention further provides **peptides** which include cell-growth affecting **peptides** and **peptides** which enhance or inhibit production of cellular proteins. Many of the **peptides** of the invention may be produced in large quantity by recombinant techniques and formulated in **culture medium** to produce the desired effect on cultured cells and tissues. Certain of the **libraries** of the invention and the **peptides** identified in them are particularly useful in concatemer-based recombinant expression methods.

L4 ANSWER 5 OF 148 MEDLINE on STN

AN 2004555856 MEDLINE
DN PubMed ID: 15528568
TI Selection of single-chain antibodies against the VP8* subunit of rotavirus VP4 outer capsid protein and their expression in Lactobacillus casei.
AU Monedero Vicente; Rodriguez-Diaz Jesus; Viana Rosa; Buesa Javier; Perez-Martinez Gaspar
CS Departamento de Biotecnologia, Instituto de Agroquimica y Tecnologia de Alimentos (CSIC), Burjassot, Spain.
SO Applied and environmental microbiology, (2004 Nov) 70 (11) 6936-9. Journal code: 7605801. ISSN: 0099-2240.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200502
ED Entered STN: 20041106
Last Updated on STN: 20050301
Entered Medline: 20050228
AB Single-chain antibodies (scFv) recognizing the VP8* fraction of rotavirus outer capsid and blocking rotavirus infection in vitro were isolated by phage display. Vectors for the extracellular expression in Lactobacillus casei of one of the scFv were constructed. L. casei was able to secrete active scFv to the **growth medium**, showing the potential of probiotic bacteria to be engineered to express molecules suitable for in vivo antirotavirus therapies.

=> s l4 and py<1999
1 FILES SEARCHED...
L5 74 L4 AND PY<1999

=> d 1-10 bib ab

L5 ANSWER 1 OF 74 MEDLINE on STN
AN 2000453953 MEDLINE
DN PubMed ID: 11012204
TI Characterization, molecular cloning and expression of megakaryocyte potentiating factor.
AU Yamaguchi N; Yamamura Y; Konishi E; Ueda K; Kojima T; Hattori K; Oheda M; Imai N; Taniguchi Y; Tamura M; Ochi N
CS Department of Cell Biology, Research Institute for Neurological Diseases and Geriatrics, Kyoto Prefectural University of Medicine, Japan.
SO Stem cells (Dayton, Ohio), **(1996)** 14 Suppl 1 62-74. Journal code: 9304532. ISSN: 1066-5099.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200011
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001102
AB We examined whether the conditioned media of 64 kinds of cell lines, which have been maintained by a protein-free culture system, could produce megakaryocyte potentiating (Meg-POT) activity. In these cell lines, HPC-Y5, established from human pancreatic cancer, was shown to have the highest level of activity. The megakaryocyte potentiating factor (MPF) was purified from its conditioned medium by a combination of ion-exchange chromatography, gel filtration and reversed-phase HPLC. The purified MPF showed Meg-POT activity almost equal to human (Hu) interleukin 6 (IL-6) in the presence of murine IL-3 in a colony-forming assay with mouse bone marrow cells. The molecular weight of MPF was estimated to be 33 kDa by SDS-PAGE. Glycopeptidase F digestion and amino sugar analysis of the factor demonstrated that MPF is a glycoprotein carrying at least one

N-linked sugar chain. The N-terminal amino acid sequence of MPF was determined to be Leu-Ala-Gly-Glu-Thr-Gly-Gln-Glu-Ala-Ala-Pro-Leu-Asp-Gly-Val-Leu-Ala-Asn. The same or homologous amino acid sequence has not been found in known proteins, demonstrating that MPF may be a novel cytokine which has Meg-POT activity. Then, we isolated HuMPF cDNA from an HPC-Y5 cDNA **library** using polymerase chain reaction and plaque hybridization methods. The HuMPF cDNA encodes a polypeptide consisting of 622 amino acids, including a signal **peptide** of 33 amino acids, and with a deduced molecular weight of 68 kDa, although HPC-Y5 cells secrete a 33 kDa form of HuMPF. HuMPF cDNA does not show any significant homology with other known sequences. The cDNA was expressed in COS-7 and Chinese hamster ovary (CHO) cells, and Meg-POT activity was detected in their culture supernatant. The COS-7 cells secreted only a 33 kDa recombinant (r)HuMPF, however, an additional 30 kDa form was detected in the **culture medium** of CHO cells. The 33 kDa rHuMPF from CHO cells showed Meg-POT activity, but not the purified 30 kDa rHuMPF. The difference in structure and activity between the 33 and 30 kDa forms of HuMPF was ascribed to the existence in the 33 kDa form of the C-terminal 25 amino acid residues. The expression of MPF mRNA was examined by Northern blot analysis using labeled MPF cDNA as a probe. MPF mRNA was detected in HPC-Y5 cells, with an approximate molecular size of 2.4 kb. We also examined the expression of the MPF gene in various human tissues, and the 2.4 kb band was detected only in lung. Then, the immunohistocytochemical analysis and in situ hybridization revealed that MPF-producing cells were identified as lung macrophages. MPF may exhibit other biological activities such as regeneration of the lung tissues.

L5 ANSWER 2 OF 74 MEDLINE on STN
AN 1999005370 MEDLINE
DN PubMed ID: 9787179
TI Identification of four genes in endothelial cells whose expression is affected by tumor cells and host immune status--a study in ex vivo-isolated endothelial cells.
AU Liliensiek B; Rocha M; Umansky V; Benner A; Lin J; Ziegler R; Nawroth P P; Schirrmacher V
CS Department of Internal Medicine I, University of Heidelberg, Heidelberg; and the German Cancer Research Center, Tumor Immunology Program and the Division of Biostatistics, Heidelberg, Germany.
SO Blood, **(1998 Nov 1)** 92 (9) 3394-404.
Journal code: 7603509. ISSN: 0006-4971.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
OS GENBANK-AF013213; GENBANK-AF013214; GENBANK-AF013215
EM 199811
ED Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981130
AB A spontaneously metastasizing, well-defined mouse lymphoma was chosen as an in vivo model to study the effect of tumor-host interaction on gene expression in liver sinusoidal endothelial cells. Forty-nine bovine aortic endothelial cell (BAEC) genes, recently isolated by a differential screening approach of a cDNA **library** enriched for tumor necrosis factor-alpha (TNF-alpha) suppressed genes, were investigated. Four of these genes were finally selected because they were affected differentially by host immuno-competence, TNF-alpha, and tumor cells. Sequence analysis showed them to encode the bovine polyubiquitin (A4), elongation factor 1alpha (B2), the acidic ribosomal phosphoprotein PO (C3), and the ribosomal protein S2 (E10). Gene expression was analyzed by dot-blot or Northern blot analysis. TNF-alpha and tumor cell conditioned supernatant suppressed the genes additive in BAEC but not in other endothelial cells except for bovine capillary endothelial cells. Ex vivo-isolated liver endothelial cells of tumor-bearing syngeneic DBA/2

mice showed strong downregulation of these four genes in comparison to normal control values. In contrast, endothelial cells of tumor-bearing immuno-incompetent Balb/c (nu/nu) mice showed no downregulation but upregulation of these genes. Consistently, all four genes were also downregulated when BAEC were incubated with supernatants derived from ex vivo-isolated liver metastases from immuno-competent but not from -incompetent mice. Thus, the expression of a group of genes involved in protein translation and processing was more profoundly altered in endothelial cells in vivo than in vitro, suggesting that microenviromental factors and cell-cell and cell-matrix interactions play an important role.

L5 ANSWER 3 OF 74 MEDLINE on STN
AN 1998443226 MEDLINE
DN PubMed ID: 9769328
TI Cloning and identification of human Sca as a novel inhibitor of osteoclast formation and bone resorption.
AU Choi S J; Devlin R D; Menaa C; Chung H; Roodman G D; Reddy S V
CS Department of Medicine/Hematology, University of Texas Health Science Center, San Antonio, Texas 78284, USA.
NC AG13625 (NIA)
AM35188 (NIADDK)
AR41336 (NIAMS)
SO Journal of clinical investigation, **(1998 Oct 1)** 102 (7) 1360-8.
Journal code: 7802877. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals; Space Life Sciences
EM 199811
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981112
AB Increased osteoclast activity is responsible for the enhanced bone destruction in postmenopausal osteoporosis, Paget's disease, bone metastasis, and hypercalcemia of malignancy. However, the number of known inhibitory factors that block osteoclast formation and bone resorption are limited. Therefore, we used an expression-cloning approach to identify novel factors produced by osteoclasts that inhibit osteoclast activity. A candidate clone was identified and isolated from a human osteoclast-like multinucleated cell (MNC) cDNA **library**, named osteoclast inhibitory **peptide**-1 (OIP-1), and the cDNA sequence was determined. This sequence matched that of the recently identified human stem cell antigen, was structurally similar to the mouse Ly-6 gene family, and the sequence predicted it was a glycosyl phosphatidyl inositol (GPI)-anchored protein that had a cleavable COOH-terminal **peptide** . Western blot analysis of conditioned media from 293 cells transfected with the OIP-1 cDNA clone confirmed that OIP-1 was released into the media as a membrane-bound GPI-linked protein. Interestingly, both recombinant OIP-1 expressed in Escherichia coli (which does not have GPI linker) and OIP-1 expressed by mammalian cells significantly reduced osteoclast-like MNC formation induced by 1,25-dihydroxyvitamin D3 or PTH-related protein in mouse and human bone marrow cultures, and inhibited 45Ca release from prelabeled bone in fetal rat organ cultures. In contrast, recombinant OIP-1 did not inhibit the growth of a variety of other cell types. These data indicate that OIP-1 is a novel, specific inhibitor of osteoclast formation and bone resorption.

L5 ANSWER 4 OF 74 MEDLINE on STN
AN 1998411377 MEDLINE
DN PubMed ID: 9739055
TI An endothelial growth factor involved in rat renal development.
AU Oliver J A; Al-Awqati Q
CS Department of Medicine, Columbia University, College of Physicians &

Surgeons, New York, New York 10032, USA.. joa7@columbia.edu
NC DK-46934 (NIDDK)
SO Journal of clinical investigation, **(1998 Sep 15)** 102 (6) 1208-19.
Journal code: 7802877. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199810
ED Entered STN: 19981021
Last Updated on STN: 19981021
Entered Medline: 19981013
AB In the kidney, there is a close and intricate association between epithelial and endothelial cells, suggesting that a complex reciprocal interaction may exist between these two cell types during renal ontogeny. Thus, we examined whether metanephrogenic mesenchymal cells secrete endothelial mitogens. With an endothelial mitogenic assay and sequential chromatography of the proteins in the media conditioned by a cell line of rat metanephrogenic mesenchymal cells (7.1.1 cells), we isolated a protein whose amino acid analysis identified it as hepatoma-derived growth factor (HDGF). Media conditioned with Cos-7 cell transfected with HDGF cDNA stimulated endothelial DNA synthesis. With immunoaffinity purified antipeptide antibodies, we found that HDGF was widely distributed in the renal anlage at early stages of development but soon concentrated at sites of active morphogenesis and, except for some renal tubules, disappeared from the adult kidney. From a 7.1.1 cells cDNA **library**, a clone of most of the translatable region of HDGF was obtained and used to synthesize digoxigenin-labeled riboprobes. In situ hybridization showed that during kidney development mRNA for HDGF was most abundant at sites of nephron morphogenesis and in ureteric bud cells while in the adult kidney transcripts disappeared except for a small population of distal tubules. Thus, HDGF is an endothelial mitogen that is present in embryonic kidney, and its expression is synchronous with nephrogenesis.

L5 ANSWER 5 OF 74 MEDLINE on STN
AN 1998391444 MEDLINE
DN PubMed ID: 9725628
TI Selection of cadmium specific hexapeptides and their expression as OmpA fusion proteins in Escherichia coli.
AU Mejare M; Ljung S; Bulow L
CS Department of Pure and Applied Biochemistry, Centre for Chemistry and Chemical Engineering, Lund, Sweden.
SO Protein engineering, **(1998 Jun)** 11 (6) 489-94.
Journal code: 8801484. ISSN: 0269-2139.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199811
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981113
AB In searching for novel **peptides** with affinity for cadmium, the phage display technique was utilized. In the selection procedure, cadmium ions were immobilized on a metal chelating Sepharose gel. The **peptides** selected from a hexapeptide **library** showed no homology to naturally occurring metallothioneins. From the phage clones selected in the biopanning process, phages with affinity for Cd-109 in free solution were identified. The **peptide** His-Ser-Gln-Lys-Val-Phe, which was found to exhibit the strongest relative affinity for Cd-109, was cloned into Escherichia coli as a fusion to the cell surface exposed area of the outer membrane protein OmpA. Escherichia coli cells expressing this **peptide** showed increased survival in

growth media containing up to 1.2 mM CdCl2 when compared with cells not expressing this **peptide** on their surface.

L5 ANSWER 6 OF 74 MEDLINE on STN
AN 1998391047 MEDLINE
DN PubMed ID: 9725231
TI Molecular cloning and immunologic reactivity of a novel low molecular mass antigen of Mycobacterium tuberculosis.
AU Coler R N; Skeiky Y A; Vedvick T; Bement T; Ovendale P; Campos-Neto A; Alderson M R; Reed S G
CS Department of Pathobiology, University of Washington, Seattle 98195, USA.
NC AI25038 (NIAID)
GM08347 (NIGMS)
SO Journal of immunology (Baltimore, Md. : 1950), **(1998 Sep 1)** 161 (5) 2356-64.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199809
ED Entered STN: 19980917
Last Updated on STN: 19980917
Entered Medline: 19980910
AB Polypeptide Ags present in the culture filtrate of Mycobacterium tuberculosis were purified and evaluated for their ability to stimulate PBMC from purified protein derivative (PPD)-positive healthy donors. One such Ag, which elicited strong proliferation and IFN-gamma production, was further characterized. The N-terminal amino acid sequence of this polypeptide was determined and used to design oligonucleotides for screening a recombinant M. tuberculosis genomic DNA **library**. The gene (Mtb 8.4) corresponding to the identified polypeptide was cloned, sequenced, and expressed in Escherichia coli. The predicted m.w. of the recombinant protein without its signal **peptide** was 8.4 kDa. By Southern analysis, the DNA encoding this mycobacterial protein was found in the M. tuberculosis substrains H37Rv, H37Ra, Erdman, and "C" strain, as well as in certain other mycobacterial species, including Mycobacterium avium and Mycobacterium bovis BCG (bacillus Calmette-Guerin, Pasteur). The Mtb 8.4 gene appears to be absent from the environmental mycobacterial species examined thus far, including Mycobacterium smegmatis, Mycobacterium gordonae, Mycobacterium chelonae, Mycobacterium fortuitum, and Mycobacterium scrofulaceum. Recombinant Mtb 8.4 Ag induced significant proliferation as well as production of IFN-gamma, IL-10, and TNF-alpha, but not IL-5, from human PBMC isolated from PPD-positive healthy donors. Mtb 8.4 did not stimulate PBMC from PPD-negative donors. Furthermore, immunogenicity studies in mice indicate that Mtb 8.4 elicits a Th1 cytokine profile, which is considered important for protective immunity to tuberculosis. Collectively, these results demonstrate that Mtb 8.4 is an immunodominant T cell Ag of M. tuberculosis.

L5 ANSWER 7 OF 74 MEDLINE on STN
AN 1998204920 MEDLINE
DN PubMed ID: 9535912
TI Molecular characterization and expression of heparan-sulfate 6-sulfotransferase. Complete cDNA cloning in human and partial cloning in Chinese hamster ovary cells.
AU Habuchi H; Kobayashi M; Kimata K
CS Institute for Molecular Science of Medicine, Aichi Medical University, Nagakute, Aichi 480-11, Japan.
SO Journal of biological chemistry, **(1998 Apr 10)** 273 (15) 9208-13.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Priority Journals
OS GENBANK-AB006179; GENBANK-AB006180
EM 199805
ED Entered STN: 19980520
Last Updated on STN: 19980520
Entered Medline: 19980514
AB Heparan-sulfate 6-sulfotransferase (HS6ST) catalyzes the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate to position 6 of the N-sulfoglucosamine residue of heparan sulfate. The enzyme was purified to apparent homogeneity from the serum-free **culture medium** of Chinese hamster ovary (CHO) cells (Habuchi, H., Habuchi, O., and Kimata, K. (1995) J. Biol Chemical 270, 4172-4179). From the amino acid sequence data of the purified enzyme, degenerate oligonucleotides were designed and used as primers for the reverse transcriptase-polymerase chain reaction using poly(A)+ RNA from CHO cells as a template. The amplified cDNA fragment was then used as a probe to screen a cDNA **library** of CHO cells. The cDNA clone thus obtained encoded a partial **peptide** sequence composed of 236 amino acid residues that included the sequences of six **peptides** obtained after endoproteinase digestion of the purified enzyme. This cDNA clone was applied to the screening of a human fetal brain cDNA **library** by cross-hybridization. The isolated cDNA clones contained a whole open reading frame that predicts a type II transmembrane protein composed of 401 amino acid residues. No significant amino acid sequence identity to any other proteins, including heparan-sulfate 2-sulfotransferases, was observed. When the cDNA for the entire coding sequence of the protein was inserted into a eukaryotic expression vector and transfected into COS-7 cells, the HS6ST activity increased 7-fold over the control. The FLAG fusion protein purified by anti-FLAG affinity chromatography showed the HS6ST activity alone. Northern blot analysis revealed the occurrence of a single transcript of 3.9 kilobases in both human fetal brain and CHO cells. The results, together with the ones from our recent cDNA analysis of heparan-sulfate 2-sulfotransferase (Kobayashi, M., Habuchi, H., Yoneda, M., Habuchi, O., and Kimata, K. (1997) J. Biol. Chemical 272, 13980-13985), suggest that at least two different gene products are responsible for 6- and 2-O-sulfations of heparan sulfate.

L5 ANSWER 8 OF 74 MEDLINE on STN
AN 1998132622 MEDLINE
DN PubMed ID: 9465047
TI A **combinatorial** approach to the discovery of efficient cationic peptoid reagents for gene delivery.
AU Murphy J E; Uno T; Hamer J D; Cohen F E; Dwarki V; Zuckermann R N
CS Chiron Technologies, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608, USA.
SO Proceedings of the National Academy of Sciences of the United States of America, **(1998 Feb 17)** 95 (4) 1517-22.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199803
ED Entered STN: 19980326
Last Updated on STN: 20021218
Entered Medline: 19980319
AB A family of N-substituted glycine oligomers (peptoids) of defined length and sequence are shown to condense plasmid DNA into small particles, protect it from nuclease degradation, and efficiently mediate the transfection of several cell lines. The oligomers were discovered by screening a **combinatorial library** of cationic peptoids that varied in length, density of charge, side-chain shape, and hydrophobicity. Transfection activity and peptoid-DNA complex formation are shown to be highly dependent on the peptoid structure. The most

active peptoid is a 36-mer that contains 12 cationic aminoethyl side chains. This molecule can be synthesized efficiently from readily available building blocks. The peptoid condenses plasmid DNA into uniform particles 50-100 nm in diameter and mediates the transfection of a number of cell lines with efficiencies greater than or comparable to DMRIE-C, Lipofectin, and Lipofectamine. Unlike many cationic lipids, peptoids are capable of working in the presence of serum.

L5 ANSWER 9 OF 74 MEDLINE on STN
AN 1998113187 MEDLINE
DN PubMed ID: 9442065
TI Identification of functional domains of rat intestinal phospholipase B/lipase. Its cDNA cloning, expression, and tissue distribution.
AU Takemori H; Zolotaryov F N; Ting L; Urbain T; Komatsubara T; Hatano O; Okamoto M; Tojo H
CS Department of Molecular Physiological Chemistry, Osaka University Medical School, Japan.
SO Journal of biological chemistry, **(1998 Jan 23)** 273 (4) 2222-31.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-D63648
EM 199803
ED Entered STN: 19980312
Last Updated on STN: 19980312
Entered Medline: 19980303
AB A cDNA encoding a rat intestinal Ca(2+)-independent phospholipase B/lipase (PLB/LIP) was cloned from an ileac mucosa cDNA **library** using a probe amplified by polymerase chain reaction based on the purified enzyme's sequence. PLB/LIP consists of an NH2-terminal signal **peptide**, four tandem repeats of about 350 amino acids each, and a hydrophobic domain near the COOH terminus. The enzyme purified previously was found to be derived from the second repeat part. To examine the function of each domain, the full-length PLB/LIP, individual repeats, and a protein lacking the COOH-terminal hydrophobic stretch were expressed in COS-7 cells. The results showed that the second repeat, but not the other repeats, had all the activities (phospholipase A2, lysophospholipase, and lipase) found in the purified natural and expressed full-length enzymes, suggesting repeat 2 is a catalytic domain. The full-length enzyme was mainly present in membrane fractions and efficiently solubilized by treatment with 1% Triton X-100, but not with phosphatidylinositol-specific phospholipase C. Deletion of the COOH-terminal hydrophobic stretch caused the secretion of > 90% of synthesized PLB/LIP into **culture media**. These results suggest the hydrophobic domain is not replaced by a glycosylphosphatidylinositol anchor but serves as a membrane anchor directly. A message of the full-length PLB/LIP was abundantly expressed in the ileum and also, in a smaller, but significant amount, in the esophagus and testis. Immunohistochemistry showed that PLB/LIP is localized in brush border membranes of the absorptive cells, Paneth cells, and acrosomes of spermatid, suggesting its roles related and unrelated to intestinal digestion.

L5 ANSWER 10 OF 74 MEDLINE on STN
AN 1998037487 MEDLINE
DN PubMed ID: 9371436
TI Streptothricin biosynthesis is catalyzed by enzymes related to nonribosomal **peptide** bond formation.
AU Fernandez-Moreno M A; Vallin C; Malpartida F
CS Centro Nacional de Biotecnologia, CSIC, Campus Universidad Autonoma de Madrid, Canto Blanco, Madrid, Spain.
SO Journal of bacteriology, **(1997 Nov)** 179 (22) 6929-36.
Journal code: 2985120R. ISSN: 0021-9193.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-Y10293
EM 199712
ED Entered STN: 19980109
Last Updated on STN: 19990129
Entered Medline: 19971212
AB In a search for strains producing biocides with a wide spectrum of activity, a new strain was isolated. This strain was taxonomically characterized as Streptomyces rochei F20, and the chemical structure of the bioactive product extracted from its fermentation broth was determined to be a mixture of streptothricins. From a genomic **library** of the producer strain prepared in the heterologous host Streptomyces lividans, a 7.2-kb DNA fragment which conferred resistance to the antibiotic was isolated. DNA sequencing of 5.2 kb from the cloned fragment revealed five open reading frames (ORFs) such that ORF1, -2, -3, and -4 were transcribed in the same direction while ORF5 was convergently arranged. The deduced product of ORF1 strongly resembled those of genes involved in **peptide** formation by a nonribosomal mechanism; the ORF2 product strongly resembled that of mphA and mphB isolated from Escherichia coli, which determines resistance to several macrolides by a macrolide 2'-phosphotransferase activity; the ORF3 product had similarities with several hydrolases; and the ORF5 product strongly resembled streptothricin acetyltransferases from different gram-positive and gram-negative bacteria. ORF5 was shown to be responsible for acetyl coenzyme A-dependent streptothricin acetylation. No similarities in the databases for the ORF4 product were found. Unlike other **peptide** synthases, that for streptothricin biosynthesis was arranged as a multienzymatic system rather than a multifunctional protein. Insertional inactivation of ORF1 and ORF2 (and to a lesser degree, of ORF3) abolishes antibiotic biosynthesis, suggesting their involvement in the streptothricin biosynthetic pathway.

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=> d his

     (FILE 'HOME' ENTERED AT 14:11:01 ON 20 APR 2005)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 14:11:12 ON 20 APR 2005
L1       192006 S (CULTURE OR GROWTH)(2W) (MEDIA OR MEDIUM)
L2         1110 S L1 AND (COMBINATORIAL OR LIBRARY)
L3          205 S L2 AND PEPTIDE
L4          148 DUPLICATE REMOVE L3 (57 DUPLICATES REMOVED)
L5           74 S L4 AND PY<1999

=> s l1 and (combinatorial or (peptide (2a) library))
L6           82 L1 AND (COMBINATORIAL OR (PEPTIDE (2A) LIBRARY))

=> duplicate remove l6
DUPLICATE PREFERENCE IS 'MEDLINE, BIOSIS'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L6
L7           67 DUPLICATE REMOVE L6 (15 DUPLICATES REMOVED)

=> s l7 and py<1999
L8           11 L7 AND PY<1999

=> d 1-11 bib ab

L8   ANSWER 1 OF 11     MEDLINE on STN
AN   1998343691     MEDLINE
DN   PubMed ID: 9680097
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TI Effects of hyperthermia and granulocyte-macrophage colony-stimulating factor on the differentiation of human leukemic cell line U937.
AU Goliaei B; Deizadji A
CS Institute of Biochemistry and Biophysics, University of Tehran, Iran.. goliaei@nrcgeb.ac.ir
SO Leukemia research, **(1998 Aug)** 22 (8) 705-10.
Journal code: 7706787. ISSN: 0145-2126.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980806
AB We have studied the effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) and hyperthermia individually and in combination on the cell growth and differentiation of human monoblastic leukemia cell line U937. Several criteria were used to evaluate the differentiation of these cells, including the reduction in the plating efficiency and cell growth, the ability to phagocytize latex particles, the reduction of nitro blue tetrazolium (NBT), and development of surface antigenic markers. Hyperthermia alone was able to inhibit cell proliferation, reduce cell viability, and induce differentiation. In the range of 41-43 degrees C, the major effect of hyperthermia was cell differentiation induction as judged by above criteria. On average, hyperthermia induced differentiation in 32% of cells. GM-CSF was able to induce differentiation in 37% of U937 cells as judged by similar criteria. The combined treatment with GM-CSF and hyperthermia resulted in the differentiation of 60% of U937 cells. The extent of differentiation obtained is comparable or better than other **combinatorial** treatments using various cytokines or cytokines and chemical reagents reported before.

L8 ANSWER 2 OF 11 MEDLINE on STN
AN 1998132622 MEDLINE
DN PubMed ID: 9465047
TI A **combinatorial** approach to the discovery of efficient cationic peptoid reagents for gene delivery.
AU Murphy J E; Uno T; Hamer J D; Cohen F E; Dwarki V; Zuckermann R N
CS Chiron Technologies, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608, USA.
SO Proceedings of the National Academy of Sciences of the United States of America, **(1998 Feb 17)** 95 (4) 1517-22.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199803
ED Entered STN: 19980326
Last Updated on STN: 20021218
Entered Medline: 19980319
AB A family of N-substituted glycine oligomers (peptoids) of defined length and sequence are shown to condense plasmid DNA into small particles, protect it from nuclease degradation, and efficiently mediate the transfection of several cell lines. The oligomers were discovered by screening a **combinatorial** library of cationic peptoids that varied in length, density of charge, side-chain shape, and hydrophobicity. Transfection activity and peptoid-DNA complex formation are shown to be highly dependent on the peptoid structure. The most active peptoid is a 36-mer that contains 12 cationic aminoethyl side chains. This molecule can be synthesized efficiently from readily available building blocks. The peptoid condenses plasmid DNA into uniform particles 50-100 nm in

diameter and mediates the transfection of a number of cell lines with efficiencies greater than or comparable to DMRIE-C, Lipofectin, and Lipofectamine. Unlike many cationic lipids, peptoids are capable of working in the presence of serum.

L8 ANSWER 3 OF 11 MEDLINE on STN
AN 1998073281 MEDLINE
DN PubMed ID: 9408951
TI A **combinatorial** library for the binuclear metal center of bacterial phosphotriesterase.
AU Watkins L M; Kuo J M; Chen-Goodspeed M; Raushel F M
CS Department of Chemistry, Texas A&M University, College Station 77843, USA.
NC GM 33894 (NIGMS)
T32 GM 08523 (NIGMS)
SO Proteins, **(1997 Dec)** 29 (4) 553-61.
Journal code: 8700181. ISSN: 0887-3585.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199802
ED Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980210
AB Phosphotriesterase (PTE) is a zinc metalloenzyme that catalyzes the hydrolysis of an extensive array of organophosphate pesticides and mammalian acetylcholinesterase nerve agents. Although the three-dimensional crystal structure of PTE has been solved (M. M. Benning et al., Biochemistry 34:7973-7978, 1995), the precise functions of the individual amino acid residues that interact directly with the substrate at the active site are largely unknown. To construct mutants of PTE with altered specificities for particular target substrates, a simple methodology for generating a library of mutants at specific sites was developed. In this investigation, four of the six protein ligands to the binuclear metal site (His-55, His-57, His-201, and His-230) were targeted for further characterization and investigation. Using the polymerase chain reaction (PCR) protocols, a library of modified PTE genes was generated by simultaneously creating random combinations of histidine and cysteine codons at these four positions. The 16 possible DNA sequences were isolated and confirmed by dideoxy-DNA sequencing. The 16 mutant proteins were expressed in Escherichia coli and grown with the presence or absence of 1 mM CoCl2, ZnSO4, or CdSO4 in the **growth medium**. When grown in the presence of CoCl2, the H57C protein cell lysate showed greater activity for the hydrolysis of paraoxon than the wild type PTE cell lysate. H201C and H230C exhibited up to 15% of the wild-type activity, while H55C, a green protein, was inactive under all assay conditions. All other mutants had < 10(-5) of wild-type activity. None of the purified mutants that exhibited catalytic activity had a significantly altered Km for paraoxon.

L8 ANSWER 4 OF 11 MEDLINE on STN
AN 97381312 MEDLINE
DN PubMed ID: 9238644
TI Selection of a histidine-containing inhibitor of gelatinases through deconvolution of **combinatorial** tetrapeptide libraries.
AU Ferry G; Boutin J A; Atassi G; Fauchere J L; Tucker G C
CS Department of Tumour Biology, Institut de Recherches Servier, Suresnes, France.
SO Molecular diversity, **(1997)** 2 (3) 135-46.
Journal code: 9516534. ISSN: 1381-1991.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199709
ED Entered STN: 19971008
Last Updated on STN: 20030123
Entered Medline: 19970922
AB A fully automated peptide synthesizer was used to generate tetrapeptide sublibraries from 24 natural and nonnatural amino acids, from which new inhibitors of gelatinases (matrix metalloproteinases MMP-2 and MMP-9) were selected as potential anticancer drugs. MMP-2 and MMP-9 from mouse Balbc/3T3 fibroblasts conditioned media were assayed in their linear range response by zymography to quantify inhibition at each step of the tetrapeptide library deconvolution. The histidine-epsilon-amino caproic acid-beta-alanine-histidine (His-epsilon Ahx-beta Ala-His) sequence was found to yield optimal inhibition of both MMP-2 and MMP-9. Inhibition by selected tetrapeptides was also evaluated with two other techniques, a native type IV collagen degradation assay and a fluorogenic enzymatic assay, confirming the tetrapeptide potency. The His-epsilon Ahx-beta Ala-His tetrapeptide also inhibited purified human MMP-2 and MMP-9 and the corresponding enzymes present in conditioned media from human tumour cells. Finally, the length of the spacer between the two terminal histidines was found to be crucial to the inhibitory potential. This approach may thus be considered as a-successful strategy to yield specific peptide or pseudopeptide inhibitors, although their potency remains moderate, since it was measured before any chemical optimization was undertaken.

L8 ANSWER 5 OF 11 MEDLINE on STN
AN 97381302 MEDLINE
DN PubMed ID: 9238634
TI High-volume cellular screening for anticancer agents with **combinatorial** chemical libraries: a new methodology.
AU Salmon S E; Liu-Stevens R H; Zhao Y; Lebl M; Krchnak V; Wertman K; Sepetov N; Lam K S
CS Arizona Cancer Center, University of Arizona, Tucson 85724, USA.
NC CA-17094 (NCI)
CA-23074 (NCI)
UO-1 CA-57723 (NCI)
SO Molecular diversity, **(1996 Oct)** 2 (1-2) 57-63.
Journal code: 9516534. ISSN: 1381-1991.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199708
ED Entered STN: 19970902
Last Updated on STN: 19970902
Entered Medline: 19970821
AB A single-step cancer cell cytotoxic assay system for anticancer drug discovery has been developed which facilitates rapid screening of large **combinatorial** chemical libraries synthesized using the 'one-bead-one-compound' (OBOC) methodology. Each OBOC library bead incorporates two orthogonally cleavable linkers that release the bead-bound compound at a different pH. The assay utilizes high concentrations of tumor cells mixed directly with OBOC beads and plated in soft agarose containing tissue **culture medium**. One of the orthogonal linkers is cleaved at neutral pH in tissue culture releasing an aliquot of compound to diffuse at a relatively high local concentration into the soft agarose immediately surrounding the bead. Active compounds are identified visually from a clear ring of tumor cell lysis which forms within 48 h around just the rare bead releasing a cytotoxic compound. The bead releasing a cytotoxin is then plucked from the agar and the remaining compound still linked to the bead can be released for structural analysis, followed by compound resynthesis and confirmatory testing. This assay system has been successfully applied to identification of lead cytotoxic compounds from model peptidic and

non-peptidic **combinatorial** chemical libraries. Use of this methodology may facilitate anticancer drug discovery.

L8 ANSWER 6 OF 11 MEDLINE on STN
AN 97332846 MEDLINE
DN PubMed ID: 9189008
TI Insight into screening immunoglobulin gene **combinatorial** libraries in a phage display vector: a tale of two antibodies.
AU Kakinuma A; Portolano S; Chazenbalk G; Rapoport B; McLachlan S M
CS Thyroid Molecular Biology Unit, Veterans' Administration Medical Centre, San Francisco, California 94121, USA.
NC DK 36182 (NIDDK)
DK48216 (NIDDK)
SO Autoimmunity, **(1997)** 25 (2) 73-84.
Journal code: 8900070. ISSN: 0891-6934.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199708
ED Entered STN: 19970902
Last Updated on STN: 19970902
Entered Medline: 19970819
AB **Combinatorial** libraries of immunoglobulin genes in "phage display" vectors are a powerful tool for obtaining antigen-specific antibody fragments. To date, this approach has been used to isolate abundant, but not rare, human autoantibodies of IgG class. We have compared the relative efficiencies of panning pComb3 libraries made from intrathyroidal plasma cells for abundant human autoantibodies to thyroid peroxidase (TPO) and rare autoantibodies to the thyrotropin receptor (TSHR). TPO-specific Fab were readily obtained from a library using three different forms of recombinant antigen, (i) purified TPO, (ii) impure TPO in **culture medium** and, (iii) TPO expressed on the surface of CHO cells. In contrast, TSHR-specific Fab were not isolated. This was the case despite repeated pannings of six libraries from three optimal patients (IgG/kappa and IgG/lambda libraries for each patient). Both purified recombinant TSHR and CHO cells expressing TSHR on their surface were used. Library enrichment was observed on some screenings. However, Fab expressed by individual clones or from enriched libraries were not specific as determined by (i) binding to purified, radio-labeled antigen, (ii) FACS analysis of TSHR on intact CHO cells and, (iii) inhibition of radiolabeled TSH binding. Remarkably, in screening for both TPO- and TSHR-specific Fab, neither library enrichment nor the retention of cDNA inserts of the correct size correlated with obtaining Fab with the antigenic specificity sought. Indeed, excellent enrichment could be observed with conditioned medium from untransfected cells. Our data suggest that the key to isolating rare antibodies from phage display libraries is not the creation of vast libraries of greater diversity or even the development of more stable vectors. Rather, success in this endeavor appears to require reducing the "noise" of non-specific clones in a moderately sized library.

L8 ANSWER 7 OF 11 MEDLINE on STN
AN 97299064 MEDLINE
DN PubMed ID: 9154466
TI Cloning and expression of human V-genes derived from phage display libraries as fully assembled human anti-TNF alpha monoclonal antibodies.
AU Mahler S M; Marquis C P; Brown G; Roberts A; Hoogenboom H R
CS Department of Biotechnology, University of New South Wales, Kensington, Sydney, Australia.. s.mahler@unsw.edu.au
SO Immunotechnology : an international journal of immunological engineering, **(1997 Mar)** 3 (1) 31-43.
Journal code: 9511979. ISSN: 1380-2933.
CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199707
ED Entered STN: 19970724
Last Updated on STN: 19970724
Entered Medline: 19970717
AB BACKGROUND: With the advent of phage antibody libraries, access to completely human antibody fragments is feasible, either by direct selection from human antibody libraries, or by guided selection. After selection, Fabs and scFvs may need to be expressed as complete antibodies in mammalian cells for further characterisation, or if effector functions are required. OBJECTIVES: To rebuild and express the human anti-TNF alpha antibody Fab-P3A2 (isolated as a Fab fragment from phage display libraries by guided selection) as a fully assembled, functional human antibody (gamma-1, lambda) in Sp2/0 myeloma cells, and to perform preliminary characterisation studies of the secreted IgG1 molecule. A further objective was to investigate the kinetics of human antibody production and the stability of antibody secretion in transfectomas cultured in various media formulations. STUDY DESIGN: A tripartite strategy was employed for cloning heavy chain gene (VH)-P3 and light chain gene V lambda-A2-C lambda into mammalian cell expression vectors p alpha Lys-30 and p alpha Lys-17 respectively. The cell line P3A2.B5 was isolated after co-transfection of Sp2/0 mouse myelomas with the constructs, expanded and weaned into a protein free medium. Fully assembled Ig-P3A2 antibody was purified by Protein A affinity chromatography and characterised with respect to size of antibody chains, and affinity for human TNF alpha. Stability of secretion was investigated by extended serial sub-culture and analysis of P3A2.B5 sub-clones. Strategies of media enrichment were tested for any effect on antibody productivity by selected P3A2.B5 sub-clones. RESULTS: The cell line P3A2.B5 secreted an assembled, human antibody Ig-P3A2, with heavy and light chains of molecular weight 55 and 28 KD respectively. Equilibrium capture studies showed Ig-P3A2 to have a dissociation constant of approximately 1.5 x 10(-8) M. The mean specific productivity of the cell line increased from 1.2 pg/cell/day to 7.8 pg/cell/day by a combination of medium enrichment and serum reduction. Prolonged serial sub-culture of P3A2.B5 showed the cell line to be unstable with respect to antibody secretion. CONCLUSIONS: We have outlined a method for expression of human V genes as assembled antibodies in Sp2/0 myeloma cells. A cloning strategy for the stable expression of scFv or Fab genes isolated from phage display libraries as assembled human antibodies of the IgG1 subclass in Sp2/0 myeloma cells has been described. For maximising specific productivity of antibody-producing cell lines, supplementation of **culture media** with glucose, glutamine and amino acids increases antibody yield significantly compared to that in conventional media, indicating the latter is stoichiometrically limiting for production purposes.

L8 ANSWER 8 OF 11 MEDLINE on STN
AN 96413340 MEDLINE
DN PubMed ID: 8816501
TI **Combinatorial** regulation of the Saccharomyces cerevisiae CAR1 (arginase) promoter in response to multiple environmental signals.
AU Smart W C; Coffman J A; Cooper T G
CS Department of Microbiology and Immunology, University of Tennessee, Memphis 38163, USA.
NC GM-35642 (NIGMS)
SO Molecular and cellular biology, **(1996 Oct)** 16 (10) 5876-87.
Journal code: 8109087. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199611

ED Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961115

AB CAR1 (arginase) gene expression responds to multiple environmental signals; expression is induced in response to the intracellular accumulation of arginine and repressed when readily transported and catabolized nitrogen sources are available in the environment. Up to 14 cis-acting sites and 9 trans-acting factors have been implicated in regulated CAR1 transcription. In all but one case, the sites are redundant. To test whether these sites actually participate in CAR1 expression, each class of sites was inactivated by substitution mutations that retained the native spacing of the CAR1 cis-acting elements. Three types of sites function independently of the nitrogen source: two clusters of Abf1p- and Rap1p-binding sites, and a GC-rich sequence. Two different sets of nitrogen source-dependent sites are also required: the first consists of two GATAA-containing UASNTR sites that mediate nitrogen catabolite repression-sensitive transcription, and the second is arginine dependent and consists of three UAS1 elements that activate transcription only when arginine is present. A single URS1 site mediates repression of CAR1 arginine-independent upstream activator site (UAS) activity in the absence of arginine and the presence of a poor nitrogen source (a condition under which the inducer-independent Gln3p can function in association with the UASNTR sites). When arginine is present, the combined activity of the UAS elements overcomes the negative effects mediated by URS1. Mutation of the classes of sites either singly or in combination markedly alters CAR1 promoter operation and control, supporting the idea that they function synergistically to regulate expression of the gene.

L8 ANSWER 9 OF 11 MEDLINE on STN
AN 94182913 MEDLINE
DN PubMed ID: 8135514
TI Engineering and production of streptokinase in a Bacillus subtilis expression-secretion system.
AU Wong S L; Ye R; Nathoo S
CS Department of Biological Sciences, University of Calgary, Alberta, Canada.
SO Applied and environmental microbiology, **(1994 Feb)** 60 (2) 517-23.
Journal code: 7605801. ISSN: 0099-2240.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199404
ED Entered STN: 19940428
Last Updated on STN: 20000303
Entered Medline: 19940421

AB Streptokinase is one of the major blood-clot-dissolving agents used in many medical treatments. With the cloned streptokinase gene (skc) available, production of the secreted streptokinase from various Bacillus subtilis strains was studied. The use of the six-extracellular-protease-deficient strain, WB600, greatly improved the production yield of the secreted streptokinase. A modified skc which has the original skc promoter and signal sequence replaced with the B. subtilis levansucrase promoter and signal sequence was also constructed. B. subtilis carrying either the wild-type or the modified skc produces streptokinase at a comparable level. Even with WB600 as the expression host, a C-terminally-processed streptokinase was also observed. Through region-specific **combinatorial** mutagenesis around the C-terminal processing sites, streptokinase derivatives resistant to C-terminal degradation were engineered. One of the derivatives showed a 2.5-fold increase in specific activity and would potentially be a better thrombolytic agent.

L8 ANSWER 10 OF 11 MEDLINE on STN
AN 94049843 MEDLINE
DN PubMed ID: 8232337
TI Bacterially expressed Fabs of monoclonal antibodies neutralizing tumour necrosis factor alpha in vitro retain full binding and biological activity.
AU Orfanoudakis G; Karim B; Bourel D; Weiss E
CS Ecole Superieure de Biotechnologie, Strasbourg, France.
SO Molecular immunology, **(1993 Nov)** 30 (16) 1519-28.
Journal code: 7905289. ISSN: 0161-5890.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199312
ED Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931215
AB Antibody fragments specific for the human tumour necrosis factor alpha (TNF alpha) have been cloned from lambda **combinatorial** expression libraries using total RNA obtained from three different hybridoma cell lines of therapeutic interest. The previously described bacteriophage lambda vectors, lambda HC2 and lambda LC1, were modified to create unique antibody cloning sites in the **combinatorial** construct and a novel tag peptide was inserted at the C-terminal end of the expressed Fd chain. Sequence analysis of the cloned Fabs indicated that two of them were derived from a single B cell. Expression in E. coli showed that the amount of recovered Fab in the bacterial **culture medium** was related to the sequences of the variable coding regions. Hybrid Fabs created by chain exchange of similar antibodies were as active as the originally paired Fabs in binding assays. The relative affinities and the capacities of the bacterially expressed Fabs to neutralize TNF alpha cytotoxicity in vitro were identical to those of the parental antibodies. The results demonstrate that, using an in vitro approach, it is possible to generate from existing hybridoma cell lines high affinity Fabs which retain antigen specificity. The cloning sites incorporated into the C-terminal parts of these Fabs will now permit their further modification to include additional functional characteristics not possible with the original hybridoma antibodies.

L8 ANSWER 11 OF 11 MEDLINE on STN
AN 93383381 MEDLINE
DN PubMed ID: 8396803
TI Factors influencing physiological variations in the activity of the Rous sarcoma virus long terminal repeat.
AU Lang A; Fincham V J; Wyke J A
CS Beatson Institute for Cancer Research, CRC Beatson Laboratories, Bearsden, Glasgow, United Kingdom.
SO Virology, **(1993 Oct)** 196 (2) 564-75.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199310
ED Entered STN: 19931029
Last Updated on STN: 19980206
Entered Medline: 19931014
AB Previous reports have shown that Rous sarcoma virus (RSV) transcript levels in mammalian cells can be elevated by serum treatment and cellular transformation. To understand this, we have examined how the RSV long terminal repeat (LTR) enhancer is affected by cellular growth state in clonally related normal and RSV-infected Rat-1 cell lines. Functional assays with enhancer mutants have shown that two LTR CArG motifs and a

CCAAT box have individual and **combinatorial** effects on basal LTR activity, but only the CArG elements contribute to serum responses in phenotypically normal cells. Augmented enhancer activity in transformed cells is mediated in part by these CArG motifs, which under these conditions are not further stimulable by serum. Protein binding to the CArG and CCAAT elements corresponds with functional variations, binding proteins being scarce in serum-deprived normal cells and enhanced by serum stimulation, cellular transformation and, in part, cellular density. These findings provide an explanation for physiologically dependent fluctuations in RSV expression, which may include the initiation of the proviral transcriptional repression that is frequently observed in mammalian hosts. However, we also show that transcript levels of some integrated proviruses are independent of variations in the cell's ability to support LTR activity, showing that the site of insertion can be of overriding importance in determining proviral expression.